

***Colletotrichum chinense* sp. nov. from *Yucca gloriosa* and *C. quercicola* sp. nov. from *Quercus variabilis* in China**

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Abstract

Colletotrichum is an important plant pathogenic genus causing anthracnose on a wide range of host plants. During 2019 and 2021, *Colletotrichum* isolates were obtained during surveys of anthracnose on garden plants in China. Multi-gene phylogenetic analyses of internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), chitin synthase 1 (*chs-1*), actin (*act*) and beta-tubulin (*tub2*) sequences coupled with morphological evidence support the introduction of two novel species namely *Colletotrichum chinense* sp. nov. from *Yucca gloriosa* in Beijing and *C. quercicola* sp. nov. from *Quercus variabilis* in Shaanxi Province. Phylogenetic inference revealed that two isolates of *C. chinense* belonged to the agaves species complex and were closely related to *C. agaves*, and differed from the other species within this species complex by shorter conidia and the host association. Molecular identification showed that two isolates of *C. quercicola* formed a highly supported lineage close to *C. tanacetii* in the destructivum species complex, which could be distinguished from *C. tanacetii* by straighter conidia. In pathogenicity tests, yellow spots and orange conidial masses displayed on the inoculated *Y. gloriosa* leaves and brown spots appeared on the inoculated *Q. variabilis* leaves. In addition, *C. chinense* and *C. quercicola* were re-isolated from spots of the tested leaves of *Y. gloriosa* and *Q. variabilis*.

Keywords

Ascomycota, multigene phylogeny, new species, taxonomy

Introduction

The genus *Colletotrichum* (*Glomerellaceae*, *Glomerellales*, *Sordariomycetes*) is represented by its type species *Colletotrichum lineola* (Corda 1831; Damm et al. 2009; Hyde et al. 2020). The sexual morph of *Colletotrichum*, characterized by solitary or gregarious ascomata, 8-spored asci, and one-celled hyaline ascospores, was previously known as the genera *Gnomoniopsis* and *Glomerella* (Stoneman 1898; von Schrenk and Spaulding 1903; Marin-Felix et al. 2017). The asexual morph is characterized by acervular conidiomata, often with setae, producing cylindrical or crescent-shaped conidia, and by the formation of appressoria (Sutton 1992; Marin-Felix et al. 2017). With the implementation of “one fungus one name” nomenclature, *Colletotrichum* has been chosen to represent this genus based on priority (Réblová et al. 2016).

Previously, species of *Colletotrichum* were distinguished based on host range and a suite of morphological characteristics, especially the size and shape of conidia, appressoria, and sporulating structures (von Arx 1957; Cai et al. 2009; Hyde et al. 2009a, b). However, many taxonomic problems arose, due to few reliable and often variable morphological characters among species, and uncertain or broad host relationships (Cai et al. 2009; Hyde et al. 2009a; Cannon et al. 2012; Liu et al. 2016). Thus, many species are required taxonomic revision in order to clarify their taxonomic placement (Weir et al. 2012; Damm et al. 2014; Liu et al. 2022).

To establish a stable and natural classification system, Cai et al. (2009) recommended using a polyphasic approach, emphasizing multi-locus phylogeny in conjunction with morphology, geographical and ecological information to characterize and differentiate *Colletotrichum* species. Subsequently, many *Colletotrichum* species had been successfully identified and epitypified, resulting in a much better understanding of phylogenetic relationships of this genus (Weir et al. 2012; Damm et al. 2014). Currently, more than 1000 *Colletotrichum* epithets are listed in Index Fungorum (<http://www.indexfungorum.org>), and at least 303 species, grouped in 16 species complexes and some singleton species (Mu et al. 2021; Alizadeh et al. 2022; Liu et al. 2022; Zheng et al. 2022).

Many species of *Colletotrichum* have been identified as plant pathogens causing anthracnose on a wide range of hosts, especially in subtropical and tropical regions, leading to significant economic losses (Hyde et al. 2009a; Cannon et al. 2012; Lima et al. 2013). In addition, *Colletotrichum* species may occur as endophytes, saprobes, or opportunistic human pathogens, sometimes as latent plant pathogens, which may switch to a pathogenic lifestyle depending on the host plant, *Colletotrichum* species, and environmental conditions. (Huang et al. 2013; Rai and Agarkar 2014; Crous et al. 2016a; De Silva et al. 2017).

In the present study, by using a nucleotide basic local alignment search tool (BLASTn) analysis (Boratyn et al. 2013) of the ITS sequences, four *Colletotrichum* isolates from *Yucca gloriosa* and *Quercus variabilis* showed highest similarity lower than 98% with species in the agaves and destructivum species complex, respectively. The agaves species complex, represented by *Colletotrichum agaves* and four closely related species, occupies a monophyletic clade within this genus (Bhunjun et al. 2021;

Talhinhas and Baroncelli 2021). The destructivum species complex is a monophyletic group of *C. destructivum* and 19 closely related species that are mainly plant pathogens (Damm et al. 2014; Bhunjun et al. 2021; Talhinhas and Baroncelli 2021). Members of this species complex are serious economic pathogens, such as *C. destructivum*, *C. lentis* and *C. higginsianum* (Damm et al. 2014; Bhadauria et al. 2019; Khodaei et al. 2019). They are characterized by conidia that are slightly curved due to their unilaterally tapering ends and by the small inconspicuous acervuli with rather effuse growth that are sometimes difficult to spot on the host plants (Damm et al. 2014).

Recently, we investigated the phylogenetic diversity of *Colletotrichum* species associated with anthracnose on garden plants in China. Four novel isolates were collected from *Y. gloriosa* and *Q. variabilis* in Beijing and Shaanxi, respectively. The aim of this study was to identify these isolates based on phylogenetic data and morphology and to confirm their pathogenicity.

Materials and methods

Sampling and fungal isolation

From 2019 to 2021, symptomatic leaves of garden plants were collected in China. Specimens were transferred to the laboratory in paper bags and stored at 4 °C until further processing. The surface of diseased leaves were sterilized with 70% ethanol and 2% NaClO for 1 min, rinsed three times with sterile water, and then samples were cut into 0.4 × 0.4 cm small pieces excised from the margins of foliar lesions, and placed on potato dextrose agar (PDA; potato extract 20 g, dextrose 20 g, agar 20 g, 1 L distilled water) plates at 25 °C in the dark. After 2–3 days, single colonies growing from the diseased tissue were transferred to new PDA plates. Single-spore cultures were obtained from the pure colonies and examined morphologically. The cultures were deposited in the China Forestry Culture Collection Center (CFCC; <http://cfcc.caf.ac.cn/>), and the specimens in the herbarium of the Chinese Academy of Forestry (CAF; <http://museum.caf.ac.cn/>).

Morphological and culture characterisation

Agar plugs (6 mm in diameter) were taken from the edge of actively growing cultures on PDA and transferred in triplicate on PDA, synthetic low-nutrient agar (SNA; Nirenberg 1976), and malt extract agar (MEA; malt extract 20 g, agar 20 g, yeast extract 2 g, sucrose 5 g, sterile deionized water 1 L) incubated in the dark at 25 °C. After 7 days, the colony characteristics, colony diameters, and pigment production on the three media were noted. Appressoria were observed on slide cultures according to Weir et al. (2012). Moreover, the shape, color and size of conidia, conidiophores, setae, conidiogenous cells and appressoria were measured and captured at least 20 measurements using a Nikon Eclipse 80i compound microscope with differential interference contrast optics.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fungal mycelia using a CTAB DNA extraction protocol (Doyle and Doyle 1990). The internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), chitin synthase 1 (*chs-1*), actin (*act*) and beta-tubulin (*tub2*) genes were amplified and sequenced using the primer pairs ITS1/ITS4 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), CHS-79F/CHS-345R (Carbone and Kohn 1999), ACT-512F/ACT-783R (Carbone and Kohn 1999) and T1/Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997), respectively. PCR was performed in 20 µL reaction mixtures containing 10 µL 2× Taq polymerase (Tiangen, China), which contains a premix of Taq DNA polymerase (0.1 U), dNTPs (0.5 mM), MgCl₂ (3 mM) Tris-HCl (20 mM), KCl (100 mM) and the appropriate buffer system, 7 µL RNase-free water, 1 µL of each primer (0.5 µM) and 1 µL of DNA template (20 ng/µl). The PCR conditions were as follows: initial heat treatment of 5 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 s at 54 °C (ITS), 60 °C (*gapdh*), 59 °C (*chs-1*), 58 °C (*act*) or 55 °C (*tub2*), and 1 min at 72 °C, and a final elongation period of 7 min at 72 °C. Amplicons were purified and sequenced by ABI3730XL Gene Analyzer at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

Newly generated sequences from the four isolates in this study were assembled using SeqMan v. 7.1.0, and the closest match using BLASTn analyses. Reference *Colletotrichum* sequences (Table 1) were downloaded from GenBank, based on recent publication (Liu et al. 2022). Multiple sequences were aligned using the MAFFT v.7.110 online programme (<http://mafft.cbrc.jp/alignment/server/>, Katoh et al. 2019) by default settings, and adjusted manually in MEGA v.7.0 (Kumar et al. 2016). The best-fit nucleotide substitution models for each gene were selected using jModelTest v. 2.1.7 (Darriba et al. 2012) under the Akaike information criteria (AIC).

Phylogenetic analyses using Maximum Likelihood (ML) and Bayesian Inference (BI) were performed. ML analyses were constructed on the RAxML-HPG BlackBox 8.2.10 (Stamatakis 2014) using the GTR+GAMMA model with 1000 bootstrap replicates. BI analyses were also performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.2.6 (Ronquist et al. 2012). The analyses were conducted by running 5,000,000 generations in two independent runs and sampling every 100th generations. The first 25% of the trees of MCMC sampling were discarded as burn-in and posterior probabilities (PP) were determined from the remaining trees. The results were visualized in FigTree 1.4 (<http://tree.bio.ed.ac.uk/software/figtree>) and edited with Adobe Illustrator CS6.0.

Table 1. *Colletotrichum* spp. used for phylogenetic analyses in the study.

Species name	Accession no. ^a	GenBank Accession No.				
		ITS	<i>Gapdh</i>	<i>chs-1</i>	<i>act</i>	<i>tub2</i>
<i>C. agaves</i>	CBS 118190*	DQ286221	NA	NA	NA	NA
<i>C. agaves</i>	LC0947	MZ595831	MZ664053	MZ799266	MZ664129	MZ673955
<i>C. americanae-borealis</i>	ATCC 11869	KM105223	KM105578	KM105293	KM105433	KM105503
<i>C. americanae-borealis</i>	CBS 136232*	KM105224	KM105579	KM105294	KM105434	KM105504
<i>C. antirrhinicola</i>	CBS 102189*	KM105180	KM105531	KM105250	KM105390	KM105460
<i>C. attractylicola</i>	SAUCC 1307*	KR149280	KR259334	KR259333	KR132243	KU058178
<i>C. attractylicola</i>	SAUCC 130801	KU289192	KU289207	KU289202	KU289197	KU289212
<i>C. boninense</i>	CBS 123755*	JQ005153	JQ005240	JQ005327	JQ005501	JQ005588
<i>C. brasiliense</i>	CBS 128501*	JQ005235	JQ005322	JQ005409	JQ005583	JQ005669
<i>C. bryoniicola</i>	CBS 109849*	KM105181	KM105532	KM105251	KM105391	KM105461
<i>C. chinense</i>	CFCC 57501*	ON692808	ON755050	ON755046	ON755042	ON755054
<i>C. chinense</i>	CFCC 57502	ON692809	ON755051	ON755047	ON755043	ON755055
<i>C. destructivum</i>	CBS 114801	KM105219	KM105574	KM105289	KM105429	KM105499
<i>C. destructivum</i>	CBS 157.83	KM105215	KM105570	KM105285	KM105425	KM105495
<i>C. destructivum</i>	IMI 387103	KM105221	KM105576	KM105291	KM105431	KM105501
<i>C. destructivum</i>	CBS 136228*	KM105207	KM105561	KM105277	KM105417	KM105487
<i>C. euphorbiae</i>	CBS 134725*	KF777146	KF777131	KF777128	KF777125	KF777247
<i>C. fuscum</i>	CBS 133701*	KM105174	KM105524	KM105244	KM105384	KM105454
<i>C. fuscum</i>	CBS 133702	KM105178	KM105528	KM105248	KM105388	KM105458
<i>C. fuscum</i>	CBS 133703	KM105175	KM105525	KM105245	KM105385	KM105455
<i>C. fusiforme</i>	MFLUCC 12-0437	KT290266	KT290255	KT290253	KT290251	KT290256
<i>C. higginsianum</i>	CPC 19379*	KM105184	KM105535	KM105254	KM105394	KM105464
<i>C. higginsianum</i>	CPC 19364	KM105185	KM105537	KM105255	KM105395	KM105465
<i>C. higginsianum</i>	CPC 19369	KM105188	KM105540	KM105258	KM105398	KM105468
<i>C. higginsianum</i>	CPC 19394	KM105193	KM105546	KM105263	KM105403	KM105473
<i>C. ledebouriae</i>	CBS 141284*	KX228254	NA	NA	KX228357	NA
<i>C. lentis</i>	CBS 127604*	JQ005766	KM105597	JQ005787	JQ005829	JQ005850
<i>C. lentis</i>	CBS 127605	KM105241	KM105598	KM105311	KM105451	KM105521
<i>C. lini</i>	CBS 172.51*	JQ005765	KM105581	JQ005786	JQ005828	JQ005849
<i>C. lini</i>	CBS 136856	KM105233	KM105589	KM105303	KM105443	KM105513
<i>C. lini</i>	CBS 130828	KM105234	KM105590	KM105304	KM105444	KM105514
<i>C. neorubicola</i>	CCR144*	MK529906	MK547520	MK547526	MK547523	MN186400
<i>C. neorubicola</i>	CCR145	MK529908	MK547521	MK547527	MK547524	MN186401
<i>C. neorubicola</i>	CCR146	MK529907	MK547522	MK547528	MK547525	MN186402
<i>C. neosansevieriae</i>	CBS 139918*	KR476747	KR476791	NA	KR476790	KR476797
<i>C. ocimi</i>	CBS 298.94*	KM105222	KM105577	KM105292	KM105432	KM105502
<i>C. panacicola</i>	C08048	GU935867	GU935847	NA	GU944757	NA
<i>C. panacicola</i>	C08061	GU935868	GU935848	NA	GU935791	NA
<i>C. panacicola</i>	C08087	GU935869	GU935849	NA	GU944758	NA
<i>C. pisicola</i>	CBS 724.97 *	KM105172	KM105522	KM105242	KM105382	KM105452
<i>C. pleopeltidis</i>	CBS 147082*	MW883412	NA	MW890035	MW890024	NA
<i>C. quercicola</i>	CFCC 54457*	ON692810	ON755052	ON755048	ON755044	ON755056
<i>C. quercicola</i>	CFCC 57507	ON692811	ON755053	ON755049	ON755045	ON755057
<i>C. sansevieriae</i>	MAFF 239721*	LC179806	LC180130	LC180129	LC180127	LC180128
<i>C. sansevieriae</i>	BTGN2	MN386823	MN386911	NA	NA	MN386867
<i>C. shisoi</i>	JCM 31818*	MH660930	MH660931	MH660929	MH660928	MH660932
<i>C. shisoi</i>	MAFF 240106	MH660936	MH660935	MH660934	MH660933	MH660937
<i>C. tabacum</i>	CBS 124249	KM105206	KM105560	KM105276	KM105416	KM105486
<i>C. tabacum</i>	CBS 161.53	JQ005763	KM105559	JQ005784	JQ005826	JQ005847
<i>C. tabacum</i>	CPC 18945*	KM105204	KM105557	KM105274	KM105414	KM105484

Species name	Accession no. ^a	GenBank Accession No.				
		ITS	<i>Gapdh</i>	<i>chs-1</i>	<i>act</i>	<i>tub2</i>
<i>C. tanacetii</i>	BRIP 57316	JX218230	JX218245	JX259270	JX218240	JX218235
<i>C. tanacetii</i>	CBS 132693	JX218228	JX218243	JX259268	JX218238	JX218233
<i>C. tanacetii</i>	CBS 132818	JX218229	JX218244	JX259269	JX218239	JX218234
<i>C. truncatum</i>	IMI 135524	GU227874	GU228266	GU228364	GU227972	GU228168
<i>C. utrechtense</i>	CBS 130243*	KM105201	KM105554	KM105271	KM105411	KM105481
<i>C. utrechtense</i>	CBS 135827	KM105202	KM105555	KM105272	KM105412	KM105482
<i>C. utrechtense</i>	CBS 135828	KM105203	KM105556	KM105273	KM105413	KM105483
<i>C. vignae</i>	CBS 501.97*	KM105183	KM105534	KM105253	KM105393	KM105463
<i>C. vignae</i>	CPC 19383	KM105182	KM105533	KM105252	KM105392	KM105462

Notes: NA, not applicable. * ex-type strains. ^aATCC: American Type Culture Collection, Virginia, USA; BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CPC: Culture collection of Pedro Crous, housed at CBS; LC: the LC Culture Collection (a personal culture collection of Lei Cai, housed in the Institute of Microbiology, Chinese Academy of Sciences); IMI: Culture collection of CABI Europe UK Centre, Egham, UK; JCM: Japan Collection of Microorganisms, RIKEN Bioresource Center, Tsukuba, Japan; MAFF: MAFF Genbank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLU: Mae Fah Luang University Culture Collection, Thailand; SAUCC: Department of Plant Pathology, College of Plant Protection, Shenyang Agricultural University, China.

Pathogenicity test

The pathogenicity of two *Colletotrichum* isolates was assessed on detached healthy *Y. gloriosa* and *Q. variabilis* plants in the greenhouse. Leaves were washed in running distilled water, surface-sterilized in 70% ethanol and 2% NaClO for 1 min, then rinsed in sterile distilled water. Spores were harvested from two-week-old PDA plates with 10 ml of sterilized water with spore suspension filtered through two layers of cheesecloth to eliminate debris and mycelium. The conidial suspension was adjusted to a final inoculum concentration of 1 × 10⁶–10⁷ conidia/mL with sterile deionized water. Then 10 µL of conidial suspension was placed in the middle portion of the leaves, and inoculated sterile water in the additional leaves served as control. Each treatment had three replicates (three leaves), and the experiment was carried out twice. The inoculated leaves were placed in transparent plastic bags at 25 °C and over 90% humidity in the dark for 14 days. After appearance of symptoms, fungus isolates were re-isolated from the infected leaves and identified based on the morphological and phylogenetic analyses to fulfill Koch’s postulates.

Results

Phylogenetic analyses

Closest matches in BLASTn searches with the ITS sequences, these isolates were preliminarily identified to be in the agaves and destructivum species complexes. Further, phylogenetic trees were constructed based on combined loci of ITS, *gapdh*, *act*, *chs-1* and *tub2* sequences to identify these isolates to species level.

For the agaves species complex, DNA sequences of five genes were obtained from two isolates from *Y. gloriosa* in this study, with seven reference strains of the agaves species complex, and *C. boninense* (CBS 123755, ex-type) and *C. brasiliense* (CBS

128501, ex-type) as the outgroup taxa. A total of 1649 characters including alignment gaps (578 for ITS, 94 for *gapdh*, 232 for *chs-1*, 240 for *act* and 505 for *tub2*) were included in the phylogenetic analyses. Of these characters, 1271 were constant, 162 were variable and parsimony-uninformative, and 216 were parsimony-informative. The resulting ML and BI trees had similar topologies; the ML tree (Fig. 1) was selected to represent the phylogeny with ML/BI support values. Two isolates (CFCC 57501 and CFCC 57502) formed a close clade to *C. agaves* (Fig. 1).

For the destructivum species complex, DNA sequences of five genes were obtained from two isolates from *Q. variabilis* in this study, and 44 reference strains of the destructivum species complex, and *C. truncatum* (IMI 135524) and *C. fusiforme* (MFLUCC 12-0437) as the outgroup taxa. A total of 1875 characters including gaps (560 for ITS, 236 for *gapdh*, 280 for *chs-1*, 274 for *act* and 525 for *tub2*) were obtained in the phylogenetic analyses. Of

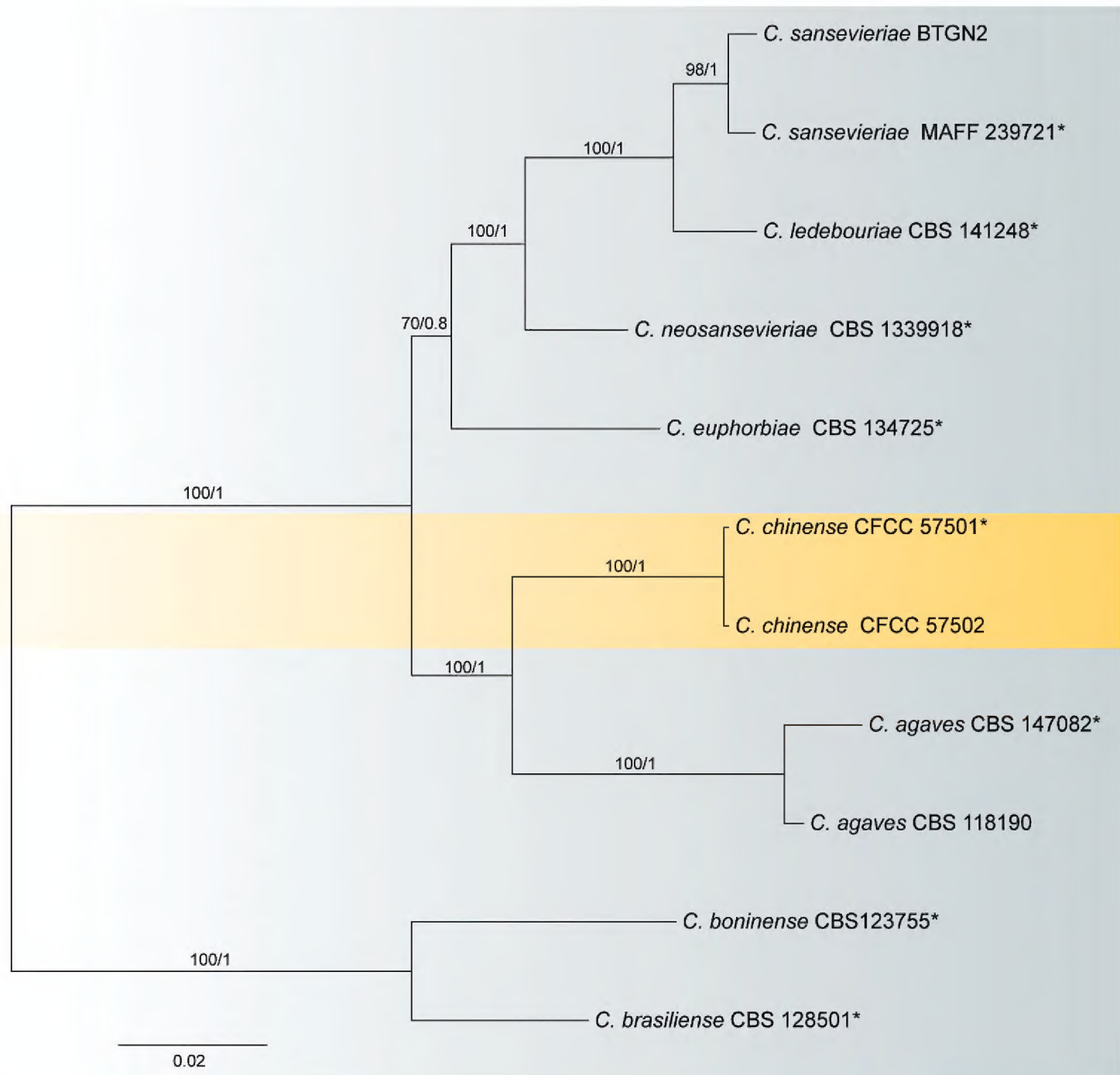


Figure 1. Phylogenetic tree obtained by Maximum likelihood analyses using the combined ITS, *gapdh*, *chs-1*, *act* and *tub2* sequence alignments of the agaves species complex. Numbers above the branches indicate ML bootstraps (left, MLBS $\geq 50\%$) and Bayesian Posterior Probabilities (right, BPP ≥ 0.7). The tree is rooted with *C. boninense* (CBS 123755, ex-type) and *C. brasiliense* (CBS 128501, ex-type).

Phylogenetic tree showing the relationships between various *Claviceps* species based on ITS1 and ITS2 sequences. The tree is rooted with *C. pleopeltidis* and *C. truncatum* as outgroups. The main clade is supported by a bootstrap value of 100/1. A yellow highlight covers the clade containing *C. tanacetii*, *C. quercicola*, and *C. lentis*. A scale bar of 0.03 is shown at the bottom left.

Species and their associated values (bootstrap/posterior probability) are listed on the right side of the tree:

- C. fuscum* CBS 133701* (70/1)
- C. fuscum* CBS 133702
- C. fuscum* CBS 133703
- C. vignae* CPC 19383 (62/1)
- C. vignae* CBS 501.97* (71/0.82)
- C. bryoniicola* CBS 109849*
- C. antirrhinicola* CBS 102189* (0/0.99)
- C. higginsianum* CPC 16396 (100/1)
- C. higginsianum* CPC 19394
- C. higginsianum* CPC 19364
- C. higginsianum* CPC 19379*
- C. panacicola* C08048 (0/0.98)
- C. panacicola* C08061 (100/1)
- C. panacicola* C08087 (97/0.98)
- C. utrechtense* CBS 135828 (86/1)
- C. utrechtense* CBS 130243*
- C. utrechtense* CBS 135827 (98/1)
- C. neorubicola* CCR144*
- C. neorubicola* CCR145
- C. neorubicola* CCR146
- C. lini* CBS 130828 (85/1)
- C. lini* CBS 136856 (93/1)
- C. lini* CBS 172.51*
- C. americae-borealis* ATCC 11869 (73/1)
- C. americae-borealis* CBS 136232* (51/0.79)
- C. destructivum* CBS 114801
- C. destructivum* CBS 136228* (90/1)
- C. destructivum* CBS 157.83 (70/0.9)
- C. destructivum* IMI 387103
- C. ocimi* CBS 298.94* (51/0.78)
- C. atractylicola* SAUCC 1307* (100/1)
- C. atractylicola* SAUCC 130801
- C. shiso* JCM 31818*
- C. shiso* MAFF 240106
- C. tabacum* CBS 161.53 (99/1)
- C. tabacum* CPC 18945* (0/0.74)
- C. tabacum* CBS 124249
- C. tanacetii* BRIP 57316 (100/1)
- C. tanacetii* CBS 132693 (100/1)
- C. tanacetii* CBS 132818 (100/1)
- C. quercicola* CFCC 57507 (99/0.97)
- C. quercicola* CFCC 54457*
- C. lentis* CBS 127604* (100/1)
- C. lentis* CBS 127605
- C. pisicola* CBS 724.97*
- C. pleopeltidis* CBS 147082*
- C. truncatum* IMI 135524 (100/1)
- C. fusiforme* MFLU MFLUCC 12-0437

Figure 2. Phylogenetic tree obtained by Maximum likelihood analyses using the combined ITS, *gapdh*, *chs-1*, *act* and *tub2* sequence alignments of the destructivum species complex. Numbers above the branches indicate ML bootstraps (left, MLBS $\geq 50\%$) and Bayesian Posterior Probabilities (right, BPP ≥ 0.7). The tree is rooted with *C. fusiforme* (MFLU 12-0437CC) and *C. truncatum* (IMI 135524).

Taxonomy

Colletotrichum chinense Ning Jiang & C.B. Wang, sp. nov.

MycoBank No: 844527

Fig. 3

Etymology. Referring to the country, where the species was first collected.

Description. *Sexual morph* not observed. *Asexual morph* developed on PDA. *Setae* and *chlamydospores* not observed. *Conidiomata* acervular, abundant, pulvinate, 200–500 µm diam. *Conidiophores* smooth-walled, unbranched, septate, sometimes constricted at the septa, hyaline, up to 40 µm long. *Conidiogenous cells* 6.5–19.5 × 3–8 µm ($\bar{x} = 12.7 \pm 2.7 \times 5.3 \pm 1.3$ µm, n = 20), subglobose to ampulliform, smooth-walled, hyaline. *Conidia* 9.5–25.5 × 3.5–8.5 µm ($\bar{x} = 14.8 \pm 1.8 \times 6 \pm 1$ µm, n = 50), L/W ratio = 2–2.7, cylindrical, obtuse at the apex, smooth-walled, hyaline, contents granular. *Appressoria* not observed.

Culture characters. Colonies on PDA, flat, with an entire margin, with sparse aerial mycelium, covered with orange conidial masses, reaching 23–25 mm diam in 7 days at 25 °C. Colonies on MEA, flat, with no aerial mycelium, covered with slimy conidial masses, reaching 15–20 diam in 7 days at 25 °C. Colonies on SNA flat, sparse white hyphae, with an entire margin, reaching 12–15 diam in 7 days at 25 °C.

Specimens examined. CHINA, Beijing City, isolated from leaf spot of *Yucca gloriosa* L., Cheng-Bin Wang, 15 August 2020 (holotype CAF800056; ex-type living culture: CFCC 57501); *Ibid* (living culture: CFCC 57502).

Notes. *Colletotrichum beeveri* of the boninense species complex and *C. tofieldiae* of the spaethianum species complex have been reported from *Yucca* before the present study (Liu et al. 2022). *Colletotrichum chinense* from the present study is similar to *C. beeveri* in the conidial shape, but differs in conidial size (9.5–25.5 × 3.5–8.5 µm in PDA vs. 12.5–15.5 × 5.5–6.5 µm in SNA) (Damm et al. 2012). In addition, *C. tofieldiae* differs from *C. chinense* by the falcate conidia (Damm et al. 2009). Based on phylogenetic analyses using multi-locus sequences (ITS, *gapdh*, *chs-1*, *act* and *tub2*), *C. chinense* formed a sister clade to *C. agaves* in the agaves species complex. The sequence identities between *C. chinense* CFCC 57501 and *C. agaves* LC0947 (21/578 ITS, 6/94 *gapdh*, 6/232 *chs-1*, 19/240 *act* and 26/505 *tub2*), *C. euphorbiae* CBS 134725 (31/578 ITS, 8/94 *gapdh*, 7/232 *chs-1*, 35/240 *act* and 32/505 *tub2*), *C. ledebouriae* CBS 141284 (29/578 ITS, 30/240 *act*), *C. neosansevieriae* CBS 139918 (28/578 ITS, 6/94 *gapdh*, 28/240 *act* and 27/416 *tub2*) and *C. sansevieriae* MAFF 239721 (29/578 ITS, 5/94 *gapdh*, 9/232 *chs-1*, 31/240 *act* and 44/505 *tub2*). (Nakamura et al. 2006; Crous et al. 2013, 2015, 2016b; Liu et al. 2022) The *chs-1* sequence of *C. neosansevieriae* CBS 139918 and the *gapdh*, *chs-1* and *tub2* sequences of *C. ledebouriae* CBS 141284 were missing. Morphologically, the conidia size of *C. chinense* are shorter than other species (Table 2).

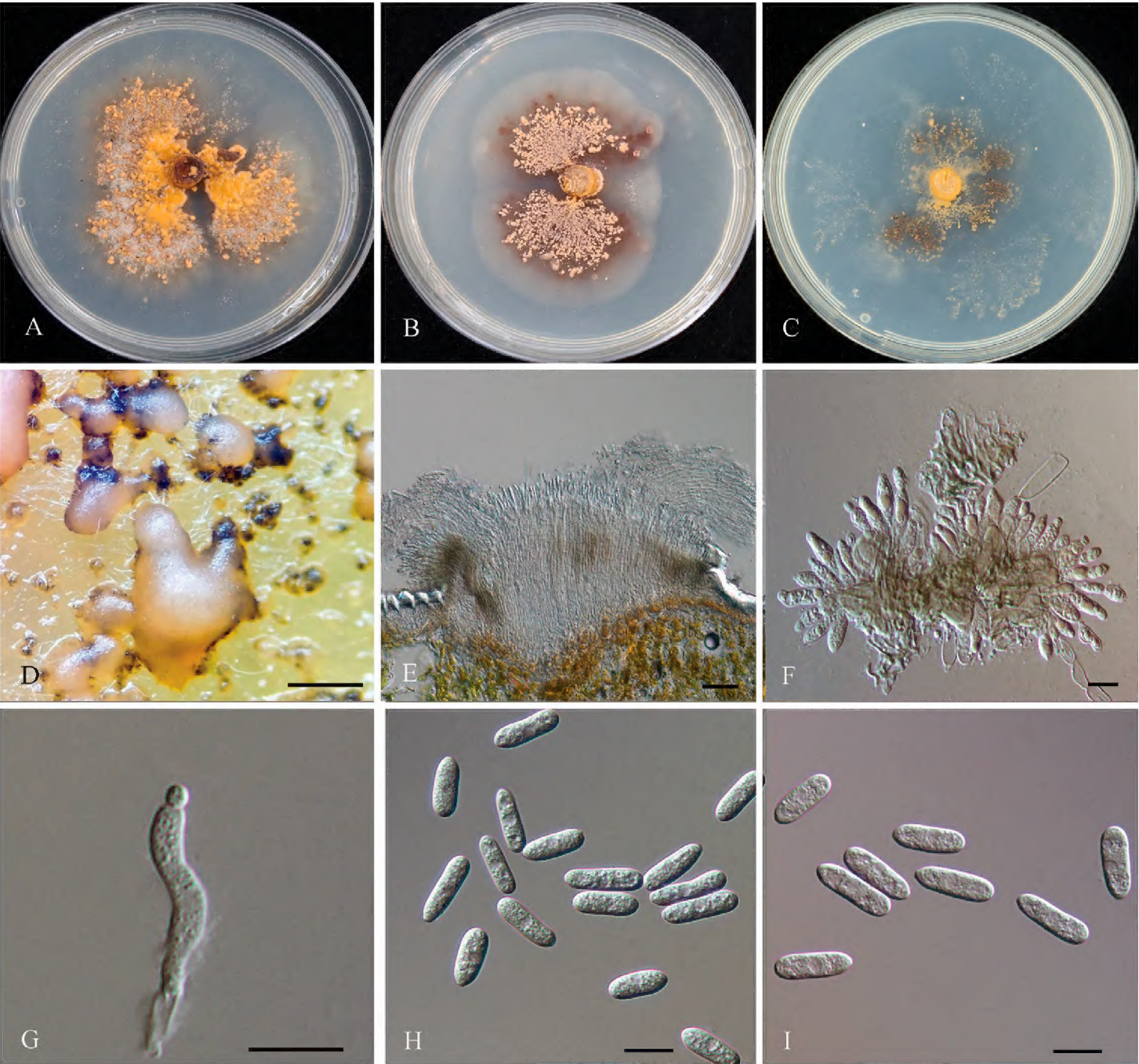


Figure 3. *Colletotrichum chinense* (CFCC 57501; ex-type) **A** colony on PDA **B** colony on MEA **C** colony on SNA **D** conidiomata formed in PDA **E** conidiophores from the host **F, G** conidiophores **H, I** conidia **F–I** from PDA. Scale bars: 200 μm (**D**); 50 μm (**E**); 20 μm (**F, G**); 10 μm (**H, I**).

Table 2. Morphological comparison of species in the agaves species complex.

Species	Type	Media for Conidia morph	Hosts	Distribution	Conidia (μm)	Appressoria (μm)	Reference
<i>C. agaves</i>	Epitype	PDA	<i>Agave</i> spp.	Mexico; USA; Netherlands	(17.5–)19.0–30.5(–33) × 5–8(–9.5) on	Not observed	Farr et al. (2006)
<i>C. chinense</i>	Holotype	PDA	<i>Yucca gloriosa</i>	China	(9.5–)12.5–16.5(–25.5) × (3.5–)6.0–7.0(–8.5)	Not observed	This study
<i>C. euphorbiae</i>	Holotype	SNA	<i>Euphorbia</i> sp	South Africa	(17–)23–28(–28.5) × (6–)6.5–7	(6.5–)8.5–14.5(–20.5) × (5.5–)6–10.5(–16)	Crous et al. (2013)
<i>C. ledebouriae</i>	Holotype	PNA	<i>Ledebouria floribunda</i>	South Africa	(15–)17–21(–22) × (5–)6	Not observed	Crous et al. (2016)
<i>C. neosansevieriae</i>	Holotype	SNA	<i>Sansevieria trifasciata</i>	South Africa	(16–)18–22(–25) × (4–)5–6	Not observed	Crous et al. (2015)
<i>C. sansevieriae</i>	Holotype	PDA	<i>Sansevieria</i> spp.	Asia; Australia; USA	12.5–(18.4)–32.5 × 3.8–(6.4)–8.8 PDA	6.3–(7.7)–8.8 × 6.3–(7.3)–7.5	Nakamura et al. (2006)

Colletotrichum quercicola Ning Jiang & C.B. Wang, sp. nov.

MycoBank No: 844528

Fig. 4

Etymology. Referring to the host genus, *Quercus*.

Description. *Sexual morph* not observed. *Asexual morph* developed on PDA. *Chlamydospores* not observed. *Conidiomata* acervular, abundant, globose to pulvinate, 200–400 µm diam. *Conidiophores*, hyaline, branched, smooth-walled, up to 50 µm long. *Setae* medium brown, smooth-walled, 60–145 µm long, 1–3-septate. *Conidiogenous cells* 6–18 × 3–7 µm ($\bar{x} = 7.9 \pm 3.6 \times 4 \pm 1.2$ µm, n = 20), hyaline, smooth-walled, cylindrical to elongate ampulliform. *Conidia* 14.5–23 × 3–5 µm ($\bar{x} = 17 \pm 1.7 \times 3.9 \pm 0.5$ µm, n = 50), L/W ratio = 4–5, hyaline, smooth-walled, fusiform, straight to slightly curved with both ends rounded or one end round and the other truncate. *Appressoria* 6–11 × 4–8 µm ($\bar{x} = 8.4 \pm 1.4 \times 5 \pm 1$ µm, n = 50), L/W ratio = 1.5–2, single, medium brown, smooth-walled, subglobose, ovate to broadly elliptical in outline.

Culture characters. Colonies on PDA flat, with moderate aerial mycelium, margin white to light gray, gray to brown in the center, reaching 46–50 mm diam in 7 days at 25 °C. Colonies on MEA flat, covered by white aerial mycelium, white margin and light orange in the center, reaching 30–35 mm diam after 7 days at 25 °C. Colonies on SNA flat, with entire margin, covered by sparse white aerial mycelium, reaching 20 mm diam after 7 days at 25 °C.

Specimens examined. CHINA, Shaanxi Province, Foping County, Dongshan Park, isolated from leaf spot of *Quercus variabilis* Bl., Yong Li, 11 September 2019 (holotype CAF800057; ex-type living culture: CFCC 54457); *Ibid* (living culture: CFCC 57507).

Notes. Four *Colletotrichum* species are presently known to occur on *Quercus* hosts, viz. *C. clidemiae*, *C. gloeosporioides*, *C. karstii* and *C. theobromicola* (Weir et al. 2012; Liu et al. 2021). *Colletotrichum quercicola* can be distinguished from those species based on any of the loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) and the fusiform conidia. *Colletotrichum quercicola* is a member of the destructivum species complex and near to *C. tanacetii*. Phylogenetically, this species can be distinguished from *C. tanacetii* CBS 132693 by 88 nucleotide differences in concatenated alignment (20/560 in ITS, 14/274 in *act*, 2/280 in *chs-1*, 17/236 in *gapdh*, and 33/525 in *tub2*) (Damm et al. 2014). Morphologically, *C. quercicola* CFCC 54457 conidia are straight to slightly curved, differing from distinctly curved conidia in *C. tanacetii* CBS 132693 (Damm et al. 2014).

Pathogenicity

Pathogenicity tests were conducted to confirm Koch's postulates on *Q. variabilis* leaves for *C. quercicola*, and on *Y. gloriosa* leaves for *C. chinense*. After 14 days of inoculation, necrotic lesions and typical orange conidial masses were observed from the inoculated site of *Y. gloriosa* leaves, and *Q. variabilis* leaves showed brown spot from the inoculated site, whereas all control leaves remained healthy (Fig. 5). Furthermore,

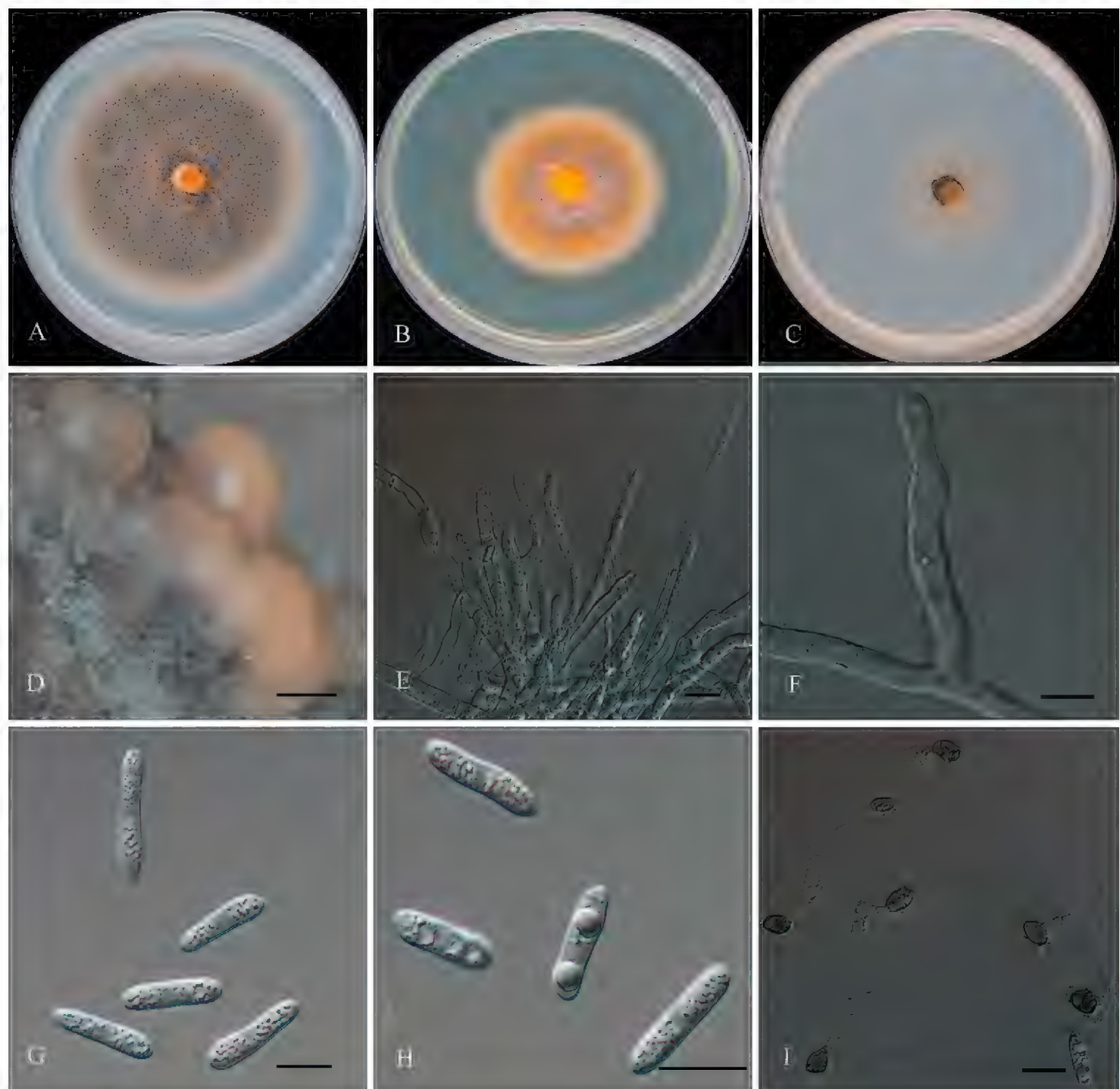


Figure 4. *Colletotrichum quercicola* (CFCC 54457; ex-type) **A** colony on PDA **B** colony on MEA **C** colony on SNA **D** conidiomata formed in PDA **E, F** conidiophores **G, H** conidia **I** appressoria were producing using a slide culture technique **E–H** from PDA. Scale bars: 200 μm (**D**); 50 μm (**E**); 20 μm (**F**); 10 μm (**G–I**).

Colletotrichum isolates could consistently be re-isolated from symptomatic lesions, but never from control leaves. And these isolates were identified as material used for inoculations based on multigene phylogenetic analyses and morphological characters, fulfilling Koch's postulates.

Discussion

In the present study, we collected garden plants with anthracnose symptoms or leaf spots in China. From these samples, the obtained *Colletotrichum* isolates were identified based on morphological features of the asexual morph obtained in culture and five combined

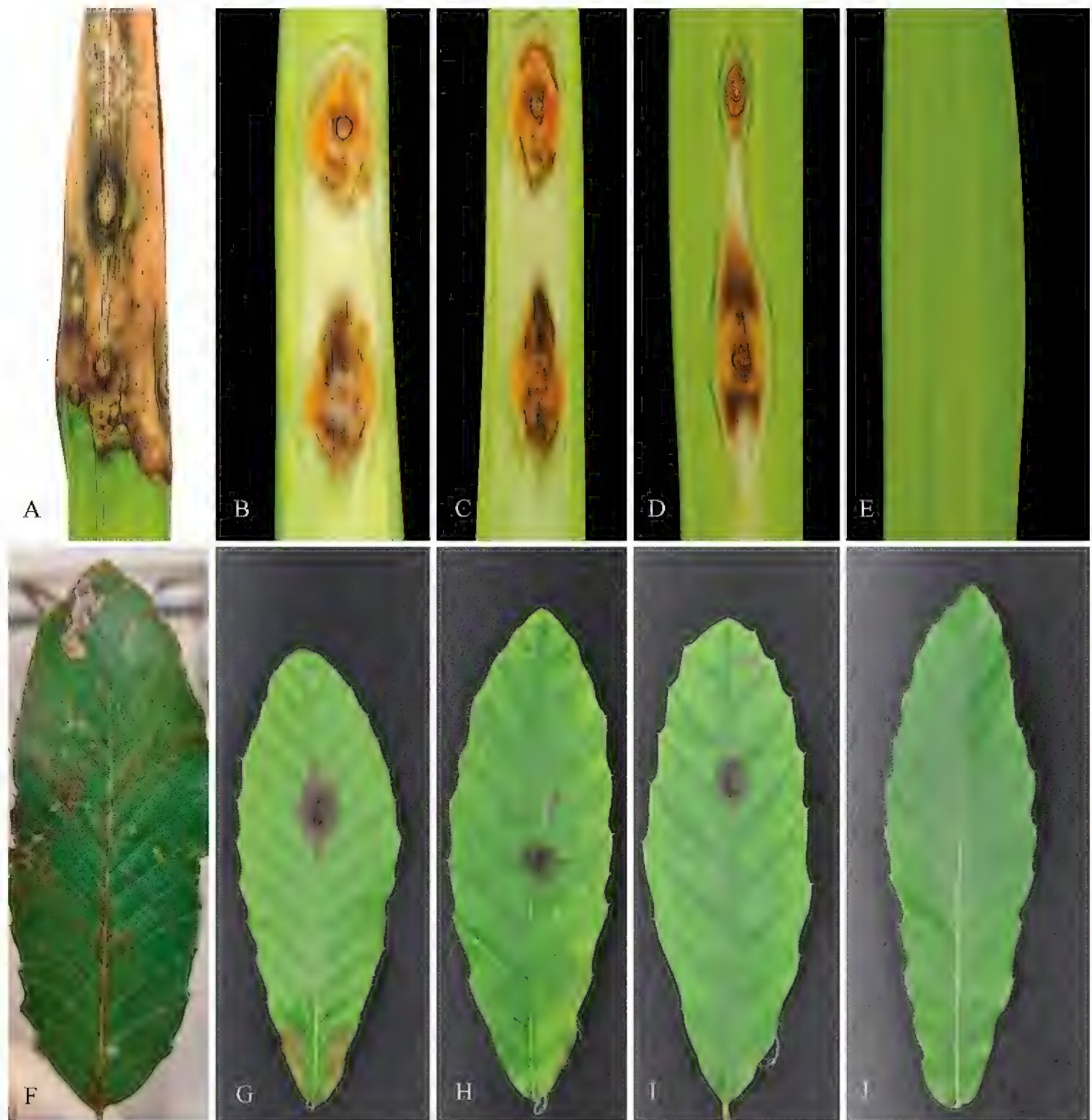


Figure 5. Typical field symptoms of disease and artificial inoculation results **A–E** *Yucca gloriosa* leaves **F–J** *Quercus variabilis* leaves **A, F** Anthracnose field symptoms **B–D** Symptoms resulting from *Colletotrichum chinense* (CFCC 57501; ex-type) after 14 days **G–I** symptoms resulting from *Colletotrichum quercicola* (CFCC 54457; ex-type) after 14 days **E, J** symptoms resulting from sterile deionized water after 14 days.

loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) phylogenies. The phylogenetic analyses revealed two novel species, *C. chinense* from *Y. gloriosa* in Beijing, and *C. quercicola* from *Q. variabilis* in the Shaanxi Province, and morphological characters can distinguish these isolates from related species. Pathogenicity test revealed *C. chinense* appearing as a causal agent of *Y. gloriosa* anthracnose and *C. quercicola* as a pathogen of *Q. variabilis* anthracnose.

ITS is evaluated as a universal DNA barcode marker for fungi (Schoch et al. 2012). However, most *Colletotrichum* species could not be distinguished based on ITS only (Cai et al. 2009; Jayawardena et al. 2016). Further, multi-locus DNA sequences, including ITS combined with supplementary barcodes, for which including some of *act*,

the intergenic region between DNA lyase and the mating-type (*mat1-2*) gene (*apMat*), DNA lyase (*apn2*), calmodulin (*cal*), *chs-1*, *gapdh*, glutamine synthetase (*gs*), superoxide dismutase (*sod2*) or *tub2* genes for species delimitation (Cannon et al. 2012; Silva et al. 2012a, b; Weir et al. 2012; Vieira et al. 2020). Generally, ITS, *act*, *chs-1*, *gapdh* and *tub2* gene regions have provided adequate resolution to differentiate species within this genus (Bhunjun et al. 2021; Jayawardena et al. 2021; Talhinhos and Baroncelli 2021). In this study, phylogenetic analyses based on five combined loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) supported that these isolates clustered in a well-supported clade in the agaves and destructivum species complexes with high confidence.

The agaves species complex groups *Colletotrichum agaves*, and four related species, *C. ledebouriae*, *C. neosansevieriae*, *C. euphorbiae* and *C. sansevieriae* (Bhunjun et al. 2021; Talhinhos and Baroncelli 2021). They are unable to be distinguished based on conidial dimensions alone (Table 2). Members of this species complex were assumed to have host specificity (Nakamura et al. 2006; Jayawardena et al. 2021; Talhinhos and Baroncelli 2021). However, three species (*C. ledebouriae*, *C. neosansevieriae* and *C. euphorbiae*) were found only once from its type strain (Crous et al. 2013, 2015, 2016b). Four species, *C. agaves* on *Agave* spp., *C. sansevieriae* on *Sansevieria* sp., *C. ledebouriae* on *Ledebouria floridunda*, *C. neosansevieriae* on *Sansevieria trifasciata*, have only been recorded from *Asparagaceae* (Talhinhos and Baroncelli 2021). In this study, *C. chinense* was isolated from symptomatic leaves of *Y. gloriosa*, belonging to the family *Asparagaceae*.

Species in the destructivum species complex are serious pathogens undergoing a hemibiotrophic lifestyle and have been associated with 49 plant species belonging to 41 genera (Damm et al. 2014; Jayawardena et al. 2021; Talhinhos and Baroncelli 2021). Many species appear to have a wide host range, while some species may affect single host species or genera (Damm et al. 2014; Talhinhos and Baroncelli 2021). Typical characteristics of species in this species complex are characterized by the presence of straight or slightly curved conidia with obtuse apices (Bhunjun et al. 2021; Jayawardena et al. 2021). Morphological differences in the size of conidia and appressoria were observed between this species complex (Table 3). The morphological approach alone makes it difficult to distinguish in this complex due to few and variable morphological characteristics.

Although morphological characters may not prove taxonomically informative for species differentiation within species complex, they are considered as a basis to taxonomic segregation for distinguishing species between different species complexes (Cannon et al. 2012; Liu et al. 2022). A polyphasic approach, emphasizing multi-gene phylogenetic analyses combined with analyses of ecological, geographical and morphological data was essential to the identification of *Colletotrichum* species (Cai et al. 2009; Jayawardena et al. 2021; Talhinhos and Baroncelli 2021). In recent years, the classification and species concepts in *Colletotrichum* was changed according to this ideal polyphasic approach (Jayawardena et al. 2021; Talhinhos and Baroncelli 2021; Liu et al. 2022). In the present study, we described two novel species based on molecular sequence analyses and morphological characters, confirming their pathological characterization. To our knowledge, this is the first report of anthracnose on *Y. gloriosa* and *Q. variabilis*. These results may provide an important basis for the prevention and control of this disease.

Table 3. Morphological comparison of species in the destructivum species complex.

Species	Type	Media for Conidia morph	Hosts	Distribution	Conidia (µm)	Appressoria (µm)	Reference
<i>C. americanae-borealis</i>	Holotype	SNA	<i>Medicago sativa</i> , <i>Glycyrrhiza uralensis</i>	America; China	(13.5–)15.5–18(–19) × 3.5–4	(4.5–)6–10.5(–13) × (3.5–)4–7(–10)	Damm et al. (2014)
<i>C. antirrhinicola</i>	Holotype	SNA	<i>Antirrhinum majus</i>	New Zealand; Japan	(14.5–)15.5–19(–23.5) × (3.5–)4–4.5(–5)	(9–)9.5–12(–13.5) × (5–)6–8(–10)	Damm et al. (2014)
<i>C. atractylodica</i>	Holotype	PDA	<i>Atractylodes lancea</i>	China	13.5–19 × 4–6.5	7.5–14 × 7–10.5	Xu et al. (2018)
<i>C. bryoniicola</i>	Holotype	SNA	genera of Asteraceae, <i>Convolvulaceae</i> , and <i>Fabaceae</i> ; etc	Netherlands; Italy	(13.5–)15–18.5(–22) × 4–5(–5.5)	(3.5–)4–10(–18) × (2.5–)3.5–6.5(–7.5)	Damm et al. (2014)
<i>C. destructivum</i>	Epitype	SNA	<i>Trifolium</i> spp.; <i>Bletilla</i> <i>ochracea</i> ; <i>Phragmites</i> sp.; etc	worldwide	(14–)14.5–16.5(–18) × 3.5–4(–4.5)	(6.5–)10–15.5(–20.5) × (4.5–)5–8(–10.5)	Damm et al. (2014)
<i>C. fuscum</i>	Epitype	SNA	<i>Digitalis</i> spp.; <i>Heracleum</i> sp.;	Germany; Italy; Netherlands	(16–)16.5–20(–34) × (3.5–)4–4.5(–5.5)	(6–)8.5–14.5(–19) × (6.5–)7–10(–11.5)	Damm et al. (2014)
<i>C. higginsianum</i>	Epitype	SNA	<i>Coreopsis lanceolata</i> <i>Brassicaceae</i> ; <i>Campanula</i> sp.;	Italy; Japan, Korea; Trinidad; Tobago; America	(17–)19–20.5(–21) × (3–)3.5–4(–4.5)	(5.5–)10–20(–28.5) × (3.5–)5–9(–12)	Damm et al. (2014)
<i>C. lenis</i>	Holotype	SNA	<i>Rumex acetosa</i>				
<i>C. lini</i>	Epitype	SNA	<i>Lens culinaris</i> ; <i>Vicia sativa</i>	Canada; China; Romania	(13–)16–20(–26) × 3–4(–5)	(5–)5.5–7.5(–9) × (3.5–)4.5–6(–6.5)	Damm et al. (2014)
<i>C. neorubicola</i>	Holotype	PDA	<i>Linum</i> sp.; <i>Nigella</i> sp.;	France; Germany; America;	(13–)15–18(–22.5) × (3–)3.5–4(–4.5)	(5–)6.5–10(–12.5) × (4–)4.5–6(–7)	Damm et al. (2014)
<i>C. ocimi</i>	Holotype	SNA	<i>Taraxacum</i> sp.; etc	Ireland; Tunisia; Netherlands			
<i>C. quercicola</i>	Holotype	PDA	<i>Rubus idaeus</i>	China	(14.8–)21.5–22.7(–23.5) × (4–)4.9–5.1(–5.6)	(4–)8.2–10.5(–17.5) × (3.6–)5.6–6.8(–11.7)	Liu et al. (2020)
<i>C. panacticola</i>	Holotype	SNA	<i>Ocimum basilicum</i>	Italy; Australia	14.5–15.5(–16.5) × (3.5–)4–4.5	(6.5–)7–13(–15.5) × (4–)4.5–7.5(–9)	Damm et al. (2014)
<i>C. pleopeltidis</i>	Holotype	SNA	<i>Quercus variabilis</i>	China	(14–)14.5–17.5(–21.5) × (3–)3.3–4.3(–4.7)	(5.7–)6.8–9.7(–10) × (3.2–)4–6(–8)	This study
<i>C. pisicola</i>	Holotype	SNA	<i>Panax</i> sp.	Eastern Asia	17.0–22.1 × 3.4–5.1	14–8	Takimoto (1919)
<i>C. shioi</i>	Holotype	SNA	<i>Pleopeltis</i> sp.	South Africa	(15–)19–23(–25) × (5–)5.5(–6)	Not described	Crous et al. (2021)
<i>C. tabacum</i>	Neotype	SNA	<i>Pisum</i> sp.	America	(11–)15–21(–29.5) × (3–)3.5–4	(5.5–)7–11.5(–13.5) × (4–)4.5–6(–6.5)	Damm et al. (2014)
			<i>Perilla frutescens</i>	Japan	(15.0–)17–19(–27.0) × (3.0–)4.0(–5.0)	(7.0–)9.0–10.0(–11.0) × (5.0–)7.0–8.0	Gan et al. (2019)
			<i>Nicotiana</i> spp., <i>Centella asiatica</i>	France; India; Germany; Madagascar; Zimbabwe	(11–5–)19–20(–27) × (3–)5–5.8(–7.6)	(10–)11.5–12.5(–14.5) × (6.5–)8.5–9.5(–11.5)	Damm et al. (2014)
<i>C. tanacetii</i>	Holotype	SNA	<i>Tanacetum cinerariifolium</i>	Australia	(13–)14.5–17.5(–19) × (3–)3.5–4(–4.5)	(5–)6.5–12(–14.6) × (3.5–)4.5–7(–10)	Damm et al. (2014)
<i>C. urechthense</i>	Holotype	PDA	<i>Trifolium pratense</i>	Netherlands	17.5–20.5(–23) × 3.5–4(–4.5)	(7–)10–14.5(–15) × (5–)6.5–9.5(–10)	Barimani et al. (2013)
<i>C. vignae</i>	Holotype	SNA	<i>Vigna unguiculata</i>	Nigeria	(12–)14–17.5(–18.5) × (3–)3.5–4(–4.5)	(4–)4.5–8.5(–12.4) × (3.5–)4–5(–6.5)	Damm et al. (2014)

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